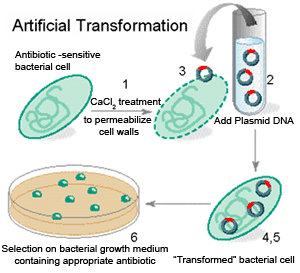
**AP Biology: Bacterial Transformation**

***How can we use genetic engineering techniques to manipulate heritable information?***

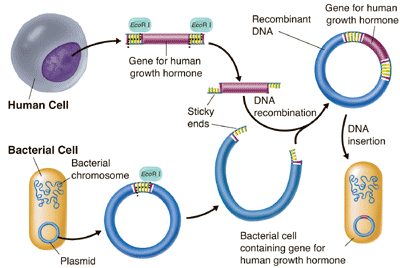
In bacteria, genetic variation does not happen by mutation alone. DNA can be introduced into bacteria three ways:

1. Conjugation: direct contact between bacterial cells in which DNA is exchanged
2. Transduction: DNA enters via a bacteriophage, virus that infects bacteria
3. **Transformation**: uptake of plasmids (“naked” DNA) from the environment surrounding bacteria

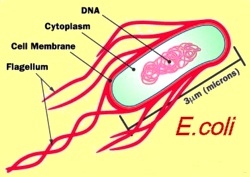
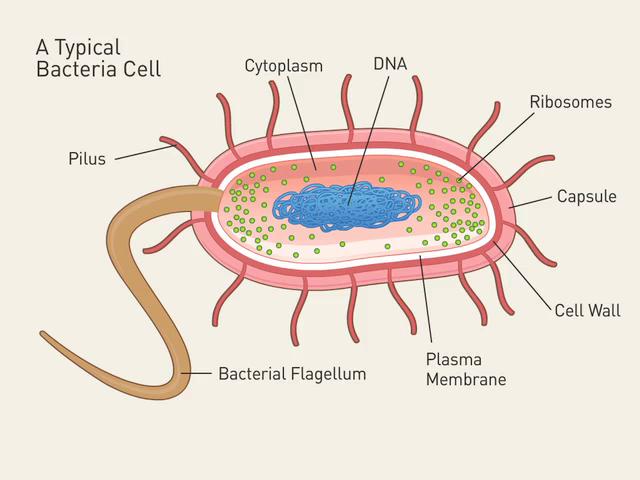
Plasmids are small, circular pieces of DNA (not part of the main DNA of a bacterium) that usually contain genes for one or more traits that may be beneficial to survival. Plasmids can code for an enzyme, toxin, or other protein that gives bacteria with that plasmid some survival advantage. In nature, bacteria may swap these beneficial plasmids from time to time. This process increases the variation between bacteria – variation that natural selection can act on.



In the laboratory, scientists use plasmids to insert “genes of interest” into an organism to change the organism’s genotype and phenotype, thus “transforming” the recipient cell. Transformation is used to make insulin and the human growth hormone. In those situation a plasmid is actually inserted with a human gene. This plasmid, known as recombinant DNA as it was recombined with another gene, is then inserted to bacteria which then reproduce. These bacteria will then produce the protein of interest, be it insulin or the human growth hormone. See the process below.

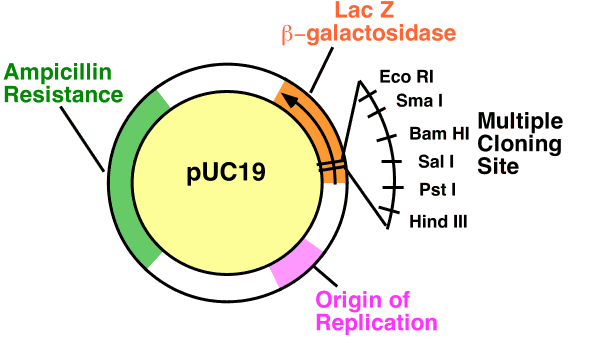


*Escherichia coli* (*E. coli*) bacteria normally live in the intestines of healthy people and animals. Most varieties of E. coli are harmless or cause relatively brief diarrhea. But a few particularly nasty strains, such as E. coli O157:H7, can cause severe abdominal cramps, bloody diarrhea and vomiting. Non-pathogenic *E. coli* is often used to study molecular biology.

*E. coli* are normally susceptible to ampicillin, an antibiotic. It affects processes involved in cell wall production.

The pUC19 plasmid will be used in this bacterial transformation. pUC19 contains both the amp-R (ampicillin resistance gene) and the lacZ gene (which codes for β-galactosidase, an enzyme which cleaves lactose into galactose and glucose).



The investigation also involves blue-white screening. Bacteria that have been transformed will be blue in the presence of IPTG and X-gal. IPTG activates the lac operon by binding to the repressor just like allolactose. However, IPTG is not broken down like allolactose therefore its concentration remains constant. X-gal is an analog of lactose and can be broken down by β-galactosidase, when this happens a blue color is produced.

This investigation provides the opportunity to review, connect, and apply the following concepts:

* Cell Structure of bacteria
* Structure and function of cell membranes, enzymes, and DNA and RNA
* Transcription and translation
* The operon model of the regulation on gene expression
* Evolution and natural selection
* Interactions between organisms and the environment

**AP Biology: Bacterial Transformation**

**Materials:**

* pUC19 plasmid
* competent (“comp”) cells = *E. coli* cells to which CaCl (calcium chloride) has been added

*must remain in ice at all times or might not work!*

* LB broth (nutrient rich medium used for bacterial growth)
* Ice bath
* Warm water bath
* Small centrifuge
* Squirt bottle of 10% bleach disinfectant
* Small test tube rack
* Marker
* Floater
* Sterile microfuge tubes
* Micropipettes of three sizes: 2-20 µl, 20-200 µl, 100-1000 µl
* Large (blue) and small (yellow) pipette tips
* Small cup for used pipette tip disposal
* Access to watch/clock/phone for timing
* Four sterile bacteriological spreaders
* Bacterial agar plates:
  1. 1 LB
  2. 2 LB/amp = ampicillin, an antibiotic has been added
  3. 1 LAX = ampicillin, X- gal, and IPTG have been added
     + X-gal = used to the presence of β-galactosidase, an enzyme produced by the lacZ gene which is part of the lac operon
     + IPTG = molecular mimic of allolactose, a lactose metabolite that triggers transcription of the lac operon

**Procedure:**

1. Transformation of Cells
2. Label one 1.5 ml microfuge tube with “N” (for “non-trans”) and your group number. Label another 1.5 ml microfuge tube with “T” (for “trans”) and your group number.
3. Using the proper micropipette, transfer 20 µl of competent cells (may need to thaw somewhat but not to room temp) to the “N” (“non-trans”) tube. Set aside on ice until step 8.
4. Using the proper micropipette, transfer 180 µl of competent cells to the “T” (“trans”) tube.
5. Using the proper micropipette, add 5 µl of the pUC19 plasmid to the “T” (“trans”) tube. When adding the plasmid make sure to add it to the bottom of the tube and directly into the competent cells and then mix the contents by gently flicking the tube. If liquid sticks to the sides of the tube, gently tap on the tabletop or spin the tubes for several seconds in the microfuge to collect the liquid at the bottom.
6. Put the “T” (“trans”) tube on ice for 15 minutes.
7. Heat shock the “T” (“trans”) tube by placing it in a floater and placing it in the 42◦C water bath for 90 seconds. *This facilitates bacterial transformation - the uptake and incorporation of foreign DNA (the plasmid)*.
8. Immediately return the “T” (“trans”) tube to the ice and leave for another 2 minutes. *Closes gaps in cell membrane.*
9. Add 800 µl of LB broth to the “T” (“trans”) tube and mix by gently pipetting several times. Incubate the tube 37◦C at (in the incubator) for 15 minutes. *This is the “recovery” period for the cells.*
10. While waiting add 180 µl of LB broth to the “N” (“non-trans”) tube to bring it up to 200 µl so it will be easier to plate. Mix well.
11. Plating of cells
12. Using a maker, label the bottom agar plates as follows:
13. An LB agar plate, with “non-trans”, your group number and the date.
14. An LB-amp agar plate, with “non-trans”, your group number and the date.
15. An LB-amp agar plate, with “trans”, your group number and the date.
16. An LAX agar plate, with “trans”, your group number and the date.
17. Pipette 100 µl of the “N” (“non-trans”) tube to the middle of the LB non-trans agar plate (plate a). Unwrap a sterile spreader and spread the liquid up and down as you rotate the plate until the entire plate is covered with the liquid. Do notthrow away the spreader.
18. Pipette 100 µl of the “N” (“non-trans”) tube to the middle of the LB/amp non-trans agar plate (plate a). Unwrap a sterile spreader and spread the liquid up and down as you rotate the plate until the entire plate is covered with the liquid. Do notthrow away the spreader.
19. Pipette 200 µl of the “T” (“trans”) tube to the middle of the LB/amp trans agar plate (plate c). Unwrap a sterile spreader and spread the liquid up and down as you rotate the plate until the entire plate is covered with the liquid. Do notthrow away the spreader.
20. Pipette 200 µl of the “T” (“trans”) tube to the middle of the LAX trans agar plate (plate D). Unwrap a sterile spreader and spread the liquid up and down as you rotate the plate until the entire plate is covered with the liquid. Do notthrow away the spreader.
21. All the liquid to soak into the plates for 10 minutes then incubate upside-down 37◦C at (in the incubator) for 24 to 48 hours. Keep the lid and bottom of the plate securely between your fingers while inverting.
22. PREDICT: On what plates will there be growth? How much? Why?

**Predictions:**

|  |  |  |
| --- | --- | --- |
| Type of environment and bacteria | Amount of growth (none, little, some, lots) | Rationale for prediction |
| LB plate with non-transformed *E. coli* |  |  |
| LB-amp plate with non-transformed *E. coli* |  |  |
| LB-amp plate with transformed *E. coli* |  |  |
| LAX plate with transformed *E. coli* |  |  |

References

AP Biology Investigative Labs: An Inquiry-Based Approach Student Manual. Investigation 8. The College Board. New York, NY

Bacterial Transformation: Ampicillin Resistance and the Lac Operon System. Carnegie Academy for Science Education. Washington, D.C.

**AP Biology: Bacterial Transformation**

**Results**

Date Table 1: Drawings of bacteria on agar plates after 48 hours

|  |  |
| --- | --- |
| LB plate with non-transformed *E. coli* | LB-amp plate with non-transformed *E. coli* |
| LB-amp plate with transformed *E. coli* | LAX plate with transformed *E. coli* |

Data Table 2: Written descriptions of results

|  |  |
| --- | --- |
| Type of environment and bacteria | Descriptions of bacteria:  What color are the bacterial colonies? How many bacterial colonies are on the plate? |
| LB plate with non-transformed *E. coli* |  |
| LB-amp plate with non-transformed *E. coli* |  |
| LB-amp plate with transformed *E. coli* |  |
| LAX plate with transformed *E. coli* |  |

**Calculating Transformation Efficiency**

Calculating transformation efficiency gives you an indication of how effective you were in getting plasmids carrying new information into host bacterial cells.

Transformation efficiency = Total number of colonies growing on the agar plate

Amount of DNA spread on the agar plate (in µg)

* + - 1. Calculate the total number of transformed cells
      2. Calculate the amount of plasmid DNA in the bacterial cells spread on the LB/amp plate